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DETERMINATION OF RESIDUE OF DPX-L5300 IN CROPS BY LIQUID CHROMATOGRAPHY

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Author

L. W. Hershberger

N. S. Heckendorn

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E. I. du Pont de Nemours & Company, Inc.
Agricultural Products Department
Research and Development Division
Experimental Station
Wilmington, Delaware 19898

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

Company E. I. du Pont de Nemours and Company, Inc.

Company Agent P. L. Friedman (Typed Name) Date 5/1/87

Registration Specialist (Title)

P. L. Friedman (Signature)

GOOD LABORATORY PRACTICE STATEMENT

The GLP requirements specified in 40 CFR Part 160 are not applicable to residue data chemistry requirements at the time of submission.

This study was conducted in the spirit of good laboratory practices.

L. W. Hershberger L W Hershberger Date 4-15-87
Study Director

Submitter E. I. du Pont de Nemours and Company, Inc.

Sponsor E. I. du Pont de Nemours and Company, Inc.

N. S. Heckendorn N S Heckendorn Date 4-15-87
Laboratory Technician Trainee

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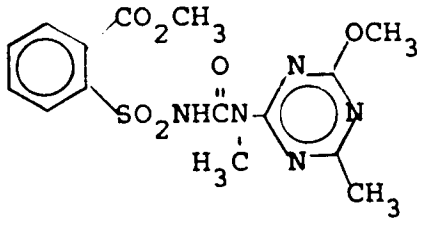
DETERMINATION OF RESIDUES OF DPX-L5300 IN CROPS BY LIQUID CHROMATOGRAPHY

ABSTRACT

A method has been developed for determination of residues of DPX-L5300 in barley and wheat grain and straw, and wheat green forage samples. The method is based on extraction of DPX-L5300 from crops with acetonitrile, and cleanup on a silica cartridge. Final determination is by normal phase liquid chromatography using a photoconductivity detector. Recoveries for 54 grain, straw and green forage samples fortified between 0.01 and 0.10 ppm averaged 88% with a standard deviation of 14%. The lower level of quantitation for grain and green forage is 0.01 ppm and for straw it is 0.02 ppm.

INTRODUCTION

Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) methylamino]carbonyl]amino]sulfonyl]benzoate (DPX-L5300)



DPX-L5300

is a Du Pont experimental sulfonylurea herbicide and is effective in controlling a variety of weeds in cereal grain fields.

A method has been developed for determination of residues of DPX-L5300 in various cereal grain crops. DPX-L5300 is extracted from crops with acetonitrile and the samples cleaned up on a silica cartridge. Final determination of DPX-L5300 is by normal phase liquid chromatography using a photoconductivity detector.

Recoveries for 54 grain, straw, and green forage samples fortified between 0.01 and 0.10 ppm averaged 88% with a standard deviation of 14%. The lower level of quantitation for grain and green forage is 0.01 ppm and for straw it is 0.02 ppm.

EQUIPMENT AND REAGENTS

A Waters liquid chromatograph equipped with a Model 590 pump, a temperature control module, a Valco Model C6W valve and a μ-Porasil, 3.9 mm i.d. x 30 cm, column was used. The liquid chromatograph and column were purchased from Waters Associates, a division of Millipore, Milford, Massachusetts. A Tracor Model 965 photoconductivity detector (Tracor Instruments, Austin, Texas) and Perkin-Elmer Model R100 recorder (Perkin-Elmer, Norwalk, Connecticut) were used for detection and display of the results.

For homogenization and extraction of samples, a Tekmar Tissumizerp® homogenizer (Tekmar Company, Cincinnati, Ohio) was used, while a Vortex-Genie® mixer (Fisher Scientific, Pittsburgh, Pennsylvania) was used for mixing of samples in centrifuge tubes.

A Millipore® all-glass filter apparatus, No. XX15 04700, with a 0.5 mm Teflon® filter, No. FHUP 047 00, was used for filtering of mobile phase, and Millipore® Millex®-SR disposable filters were used to filter samples. The Millipore® equipment was obtained from Millipore Corporation, Bedford, Massachusetts.

Final cleanup of the samples was on a silica column. Each column contained 10 g of Adsorbosil®-LC silica (Alltech Associates, Inc.; Deerfield, Illinois) packed in a 75 mL

Bond-Elut® reservoir (Analytichem International, Harbor City, California).

For concentration of samples, a vacuum rotary evaporator with a 40°C water bath and pear-shaped flasks, No. K-608700, purchased from Kontes, Vineland, New Jersey were used. An N-EVAP* evaporator, Organomation Assoc., Worcester, Massachusetts (water bath set at room temperature) concentrated the samples to final dryness under nitrogen. The Guth Universal® wash bottle was purchased from Fisher Scientific Company, Pittsburgh, Pennsylvania.

The reference standard of DPX-L5300 was obtained from the Agricultural Chemicals Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware 19898. All solvents were HPLC, distilled-in-glass grade obtained from Fisher Scientific. The chloroform was stabilized with 0.75% ethanol. All other chemicals were ACS reagent grade and were also obtained from Fisher Scientific.

PROCEDURE

Glassware Deactivation

All glassware used during the procedure was silanized to deactivate it. The silanizing reagent was prepared by mixing 30 mL of dichlorodimethyl silane and 30 mL of chlorotrimethyl silane

with 1.0 liter of toluene. The glassware was dipped in the silanizing reagent for 2 minutes and then rinsed with toluene, methanol, distilled water, and methanol respectively. After drying, the glassware was ready for use. The glassware needed to be resilanized after there was a noticeable drop in recoveries of fortified samples.

Isolation

A representative 10 gram sample of grain or wheat green forage or 5 gram sample of straw was weighed into a 250 mL glass centrifuge bottle and 120 mL of acetonitrile added.

A set of samples was sequentially homogenized with the Tissumizer® homogenizer for 1.0 minute each. Between each sample the Tissumizer® probe was rinsed with some acetonitrile from a Guth® wash bottle and the rinse added to the sample bottle. The samples were then centrifuged at 2500 rpm for 5 minutes and the liquid decanted through a glass wool plug in a funnel and collected in a 500 mL pear-shaped flask. After the first extraction of the sample set, the Tissumizer® probe was disassembled and cleaned with hot water, distilled water, and a 50:50 v/v solution of methanol and 2-propanol.

The extraction and centrifugation steps described above were repeated twice more with 100 mL acetonitrile each time. After all extractions had been combined in the pear-shaped flask, all but approximately 1 mL of acetonitrile was removed on the

vacuum rotary evaporator. The samples were then evaporated to dryness with a gentle stream of nitrogen because evaporating them to dryness on the rotary evaporator reduced recoveries.

Cleanup

For each sample, a 20 µm pore size frit was placed in the bottom of a 75 mL Bond Elute® reservoir. Ten grams of Adsorbosil®-LC silica was slurried with 50 mL of 2-propanol in a 150 mL beaker and then added to the reservoir. The beaker was rinsed with enough 2-propanol to completely transfer the silica to the reservoir. After the silica had settled, another frit was added and placed snuggly on top of the silica. Care was taken to avoid trapping air under the frit. After the 2-propanol had drained to the frit, 75 mL of chloroform was run through the column. The columns were allowed to drip under gravity but the flow was stopped whenever the solvent drained to the top frit.

Each sample was then transferred from the pear-shaped flask to a column using 3 x 5 mL rinses with chloroform. After the third rinse had drained through each column, it was rinsed with an additional 10 mL of chloroform. The column was then rinsed with 60 mL of Solution L3 (see Table 1 for composition) which was discarded along with the chloroform rinses. The DPX-L5300 was eluted with an additional 50 mL of Solution L3 which was collected in a 100 mL pear-shaped flask.

Samples were then concentrated to near dryness, (leaving

approximately 1 mL) on the rotary evaporator. Each sample was transferred from the pear-shaped flask to a 13 mL glass-stoppered centrifuge tube with 3 x 2 mL rinses of chloroform. The samples were then concentrated to dryness with the N-EVAP® evaporator and stored at ë4°C until analyzed by HPLC.

Liquid Chromatography

A Waters liquid chromatograph was used with a Perkin-Elmer recorder. A Tracor Model 965 photoconductivity detector equipped with a mercury lamp was used because of its sensitivity and selectivity for DPX-L5300. The ion exchange resin tube and pump were removed from the detector because they were not needed. The detector was further modified by placing a Nupro® metering valve (Model SS-25A-TFE) in the exit line from the reference conductivity cell. This was then adjusted to equalize the flow through the reference and analytical conductivity cells.

The HPLC mobile phase (Solution L1) was made by mixing together the volumes in Table 1 of cyclohexane, 2-propanol, and methanol in a 2.0 liter beaker. After stirring, it was filtered with a Millipore® all-glass filter apparatus using a Teflon® filter. To the filtered solution was added 1.0 mL of glacial acetic acid and 100 µL of deionized water and the solution stirred for 10 minutes. Solution L2 used for dissolving standards and samples was made the same as the mobile phase (see Table 1) except the glacial acetic acid and deionized water were not added.

A column conditioning solution (Solution L4) was made by mixing together the volumes in Table 1 of 2-propanol, methanol, glacial acetic acid, and deionized water. This solution was also filtered with the Millipore® all-glass filter apparatus using a Teflon® filter. New μ-Porasil columns were conditioned with Solution L4 for 2 hours at 1.0 mL/min and then equilibrated with mobile phase for 2 hours at the same flow rate. The conditioning procedure was also used to clean columns which lost efficiency from contamination.

For analysis of samples, an injection volume of 25 μL was used and the oven was operated at 35°C. The flow rate was set at 0.5 mL/min and the detector attentuation at 1 x 2.

A 100 μg/mL stock solution was made by dissolving 10 mg of DPX-L5300 in 100 mL of acetonitrile. A working standard (1.0 μg/mL) was prepared by pipetting 1.0 mL of the 100 μg/mL stock standard into a 100 mL volumetric flask and then diluting to volume with acetonitrile. Fortifying standards were made from the working standard at 0.1, and 0.2 μg/mL by pipetting 1.0 and 2.0 mL of the stock into 10 mL volumetric flasks and making to volume with acetonitrile.

A 1.0 μg/mL standard in Solution L2 was made by pipetting 1.0 mL of the 100 μg/mL stock standard into a 100 mL volumetric flask. The acetonitrile was removed with the N-EVAP® evaporator

and the volumetric flask made to volume with solution L2. Working HPLC standards at 0.05, 0.1, 0.2, 0.3 µg/mL were prepared weekly by pipetting 0.5, 1.0, 2.0, 3.0 mL of the 1.0 µg/mL standard into 10 mL volumetric flasks and diluting to volume with Solution L2.

Each sample was dissolved in an appropriate volume of Solution L2. To ensure complete dissolution, each sample was ultrasonically mixed, vortex mixed, and filtered with a Millex®-SR filter. Filtered samples were injected in the HPLC interspersed with standards.

Calculations

The sensitivity for each standard S, in (mm-mL)/µg units, was calculated by the equation,

$$S = \frac{(P_S)\ (A)}{C_S} \qquad (1)$$

where P_S was the peak height in millimeters, C_S was the concentration of the standard in µg/mL, and A was the attentuation. The average sensitivity, S_a, was calculated and used for calculation of sample concentrations.

The sample concentration, C, in µg/g units (ppm) was calculated using the equation:

$$C = \frac{(P)\ \ (V)}{(S_a)\ (W)} \qquad (2)$$

where P was the sample peak height in millimeters, V was the final sample volume in mL, and W was the weight of sample used in grams.

RESULTS AND DISCUSSION

This revision of AMR-337-85 had the following modifications. The column was changed from a Zorbax®-SIL column to a m-Porasil column and the mobile phase was changed from a cyclohexane, 2-propanol, acetonitrile mixture to a cyclohexane, 2-propanol, methanol mixture. These modifications should have no effect on the recovery data listed in Table 1 which were obtained without the modifications. For the m-Porasil column we currently have a sensitivity of 60mm/ng for an attentuation setting of 1 x 1.

The recoveries for 26 straw samples fortified with DPX-L5300 ranged from 65% to 118% and averaged 87%. The recoveries for 25 grain samples fortified with DPX-L5300 ranged from 70% to 112% and averaged 93%. The recoveries for 3 wheat green forage samples fortified with DPX-L5300 ranged from 75% to 85% and averaged 81%.

Chromatograms of a wheat grain control sample, the control spiked with DPX-L5300, and a treated sample are shown in Figures 1 to 3. Chromatograms of a wheat straw control sample, the control spiked with DPX-L5300, and a treated sample are shown in Figures 4 to 6.

It was determined that silanization was necessary to deactivate the glassware. If the glassware was not silanized, there was approximately a 27% loss of DPX-L5300 due to adsorption. Furthermore, silica used for the cartridges in the "clean-up" procedure may have variations between lots. Therefore, each new batch of Adsorbosil® silica should be calibrated.

TABLE 1

Solution Compositions

	Solution L1	Solution L2	Solution L3	Solution L4
Cyclohexane	780 mL	780 mL	925 mL	---
2-Propanol	110 mL	110 mL	50 mL	400 mL
Methanol	110 mL	110 mL	35 mL	400 mL
Glacial Acetic Acid	1 ml	---	5 mL	200 mL
Deionized Water	100 µL	---	50 µL	40 mL

TABLE 2

Crop	Number of Samples	Spike Range (ppm)	Recovery Range	Average Recovery	Standard Deviation (%)
Barley Grain	10	0.01 - 0.02	79% - 112%	97%	14
Barley Straw	12	0.02 - 0.04	68% - 118%	84%	13
Wheat Grain	15	0.01 - 0.02	70% - 108%	91%	12
Wheat Straw	14	0.02 - 0.04	65% - 110%	83%	14
Wheat Green Forage	3	0.01 - 0.10	75% - 85%	81%	6

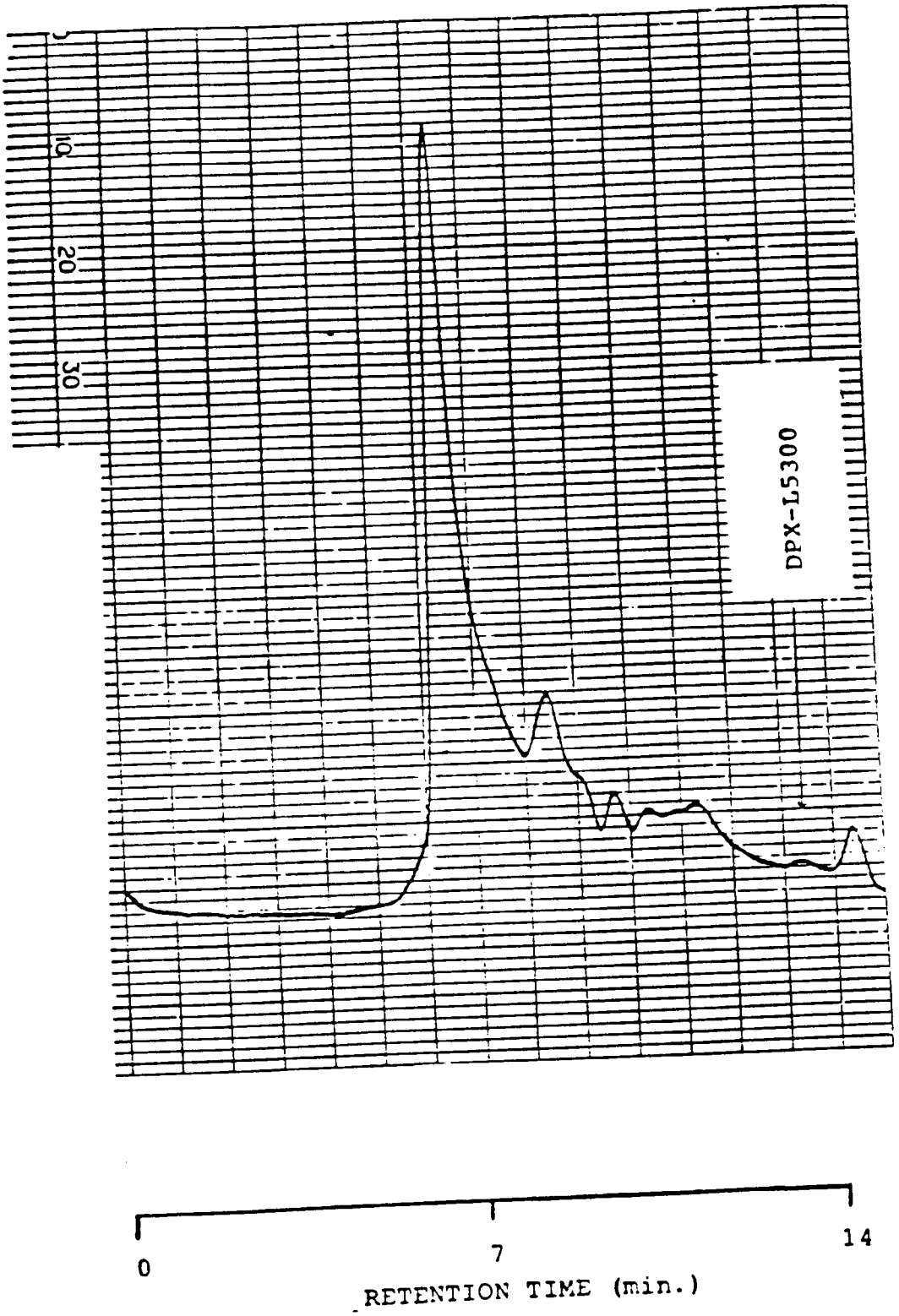


Figure 1. Chromatogram of a wheat grain control sample from The Dalles, Oregon.

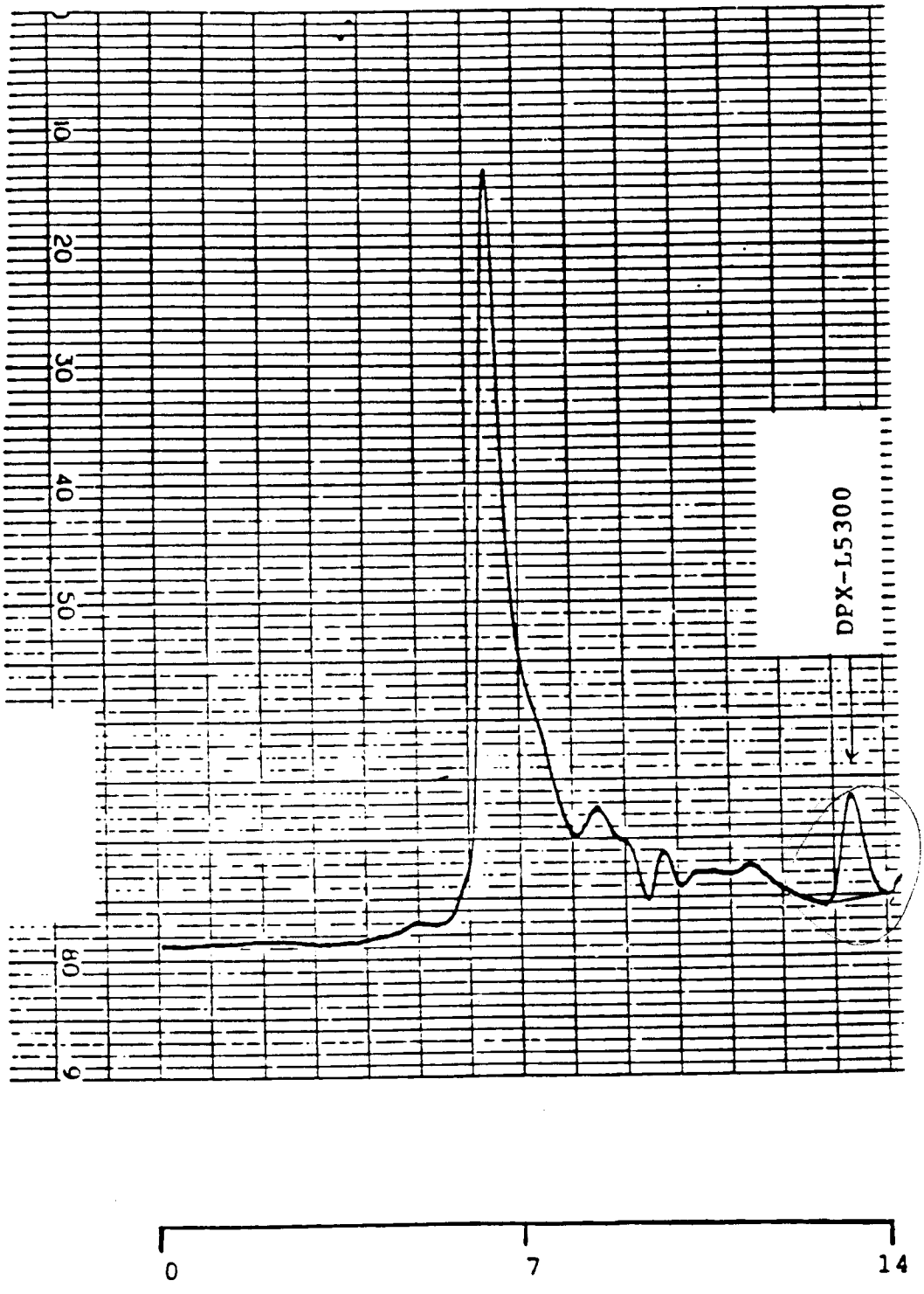


Figure 2. Chromatogram of the wheat grain control sample in Figure 1 spiked at 0.01 ppm (recovery = 93%).

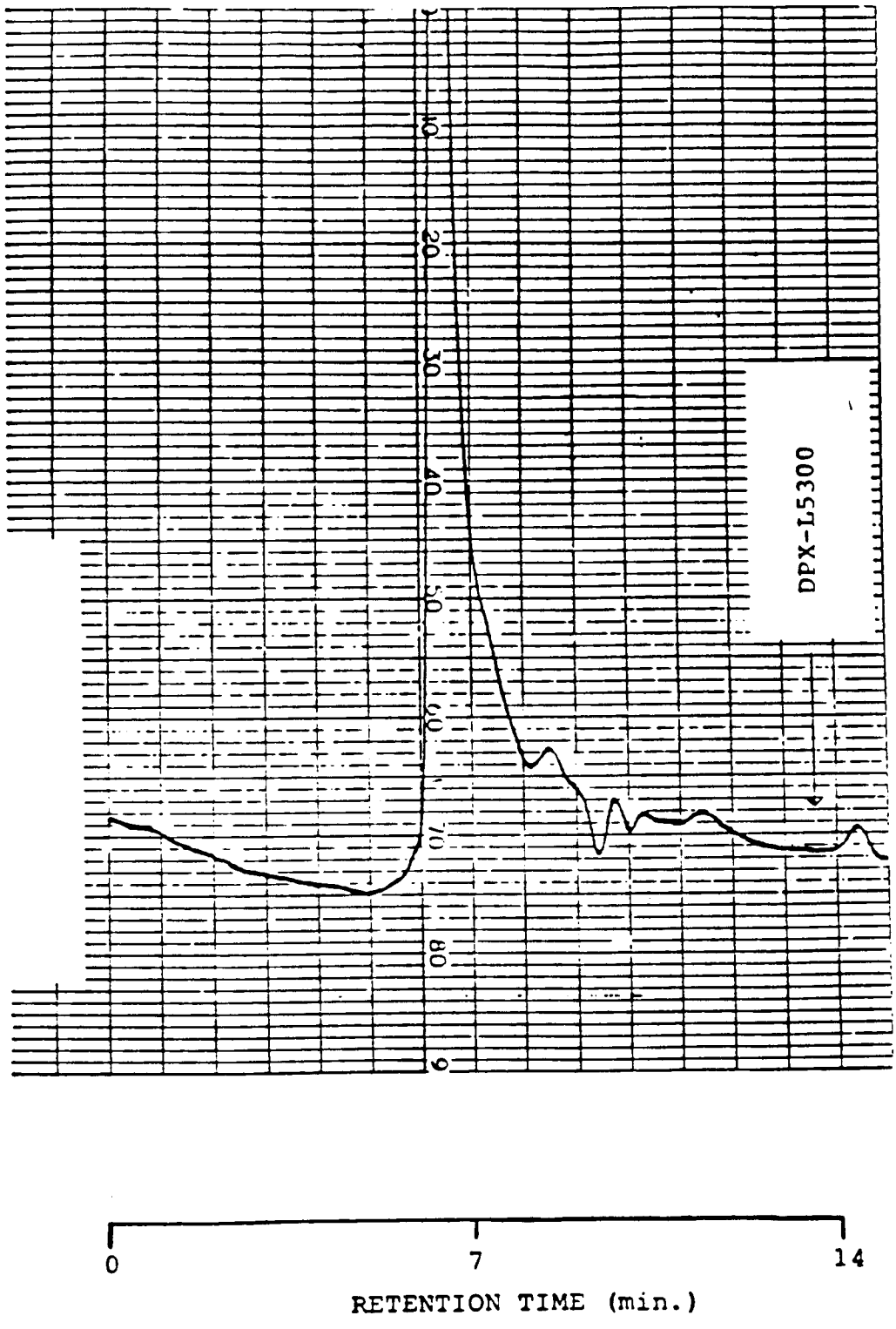


Figure 3. Chromatogram of a treated wheat grain sample (2.0 oz ai/A) from The Dalles, Oregon.

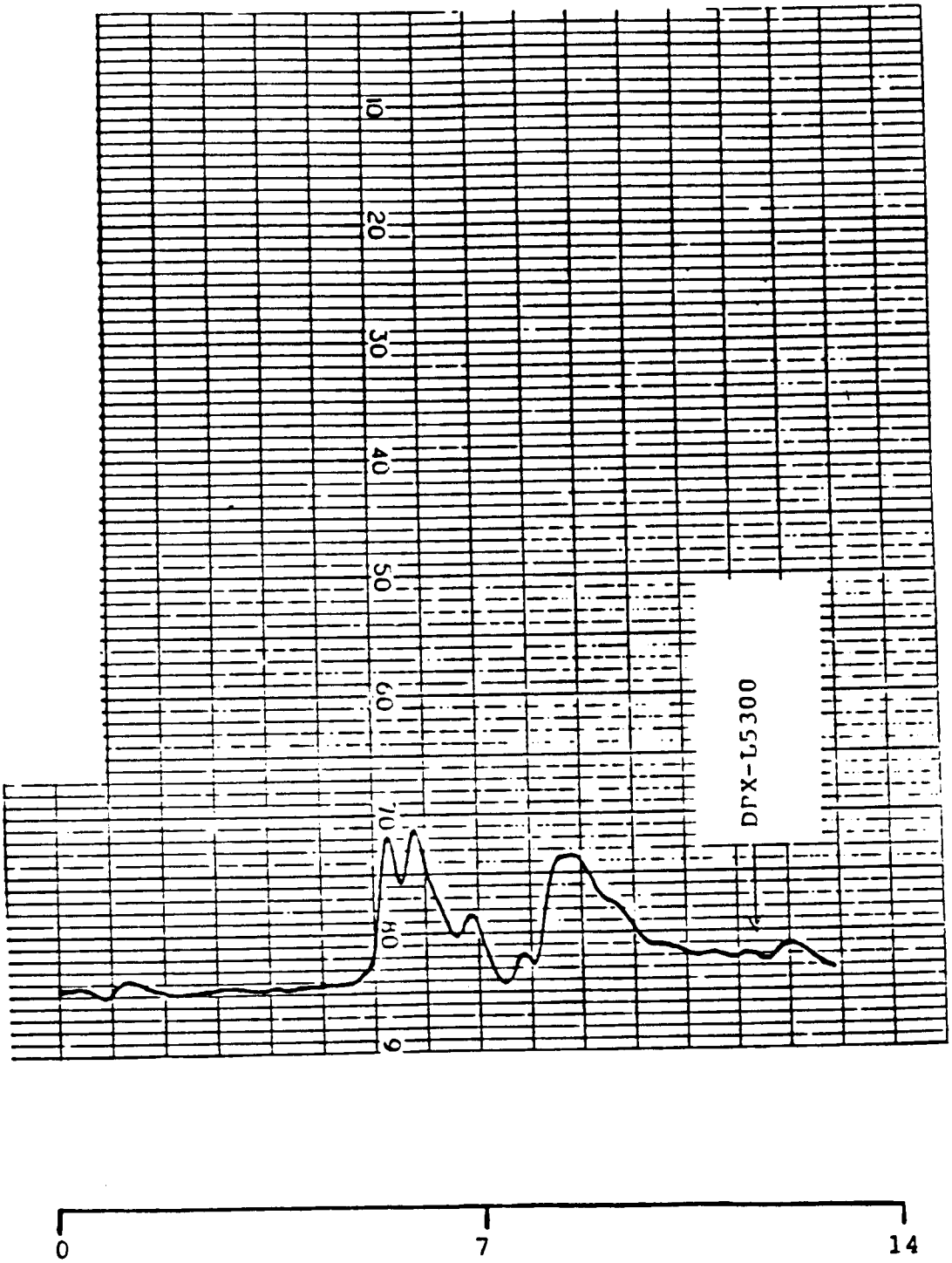


Figure 4. Chromatogram of a wheat straw control sample from McKenzie, North Dakota.

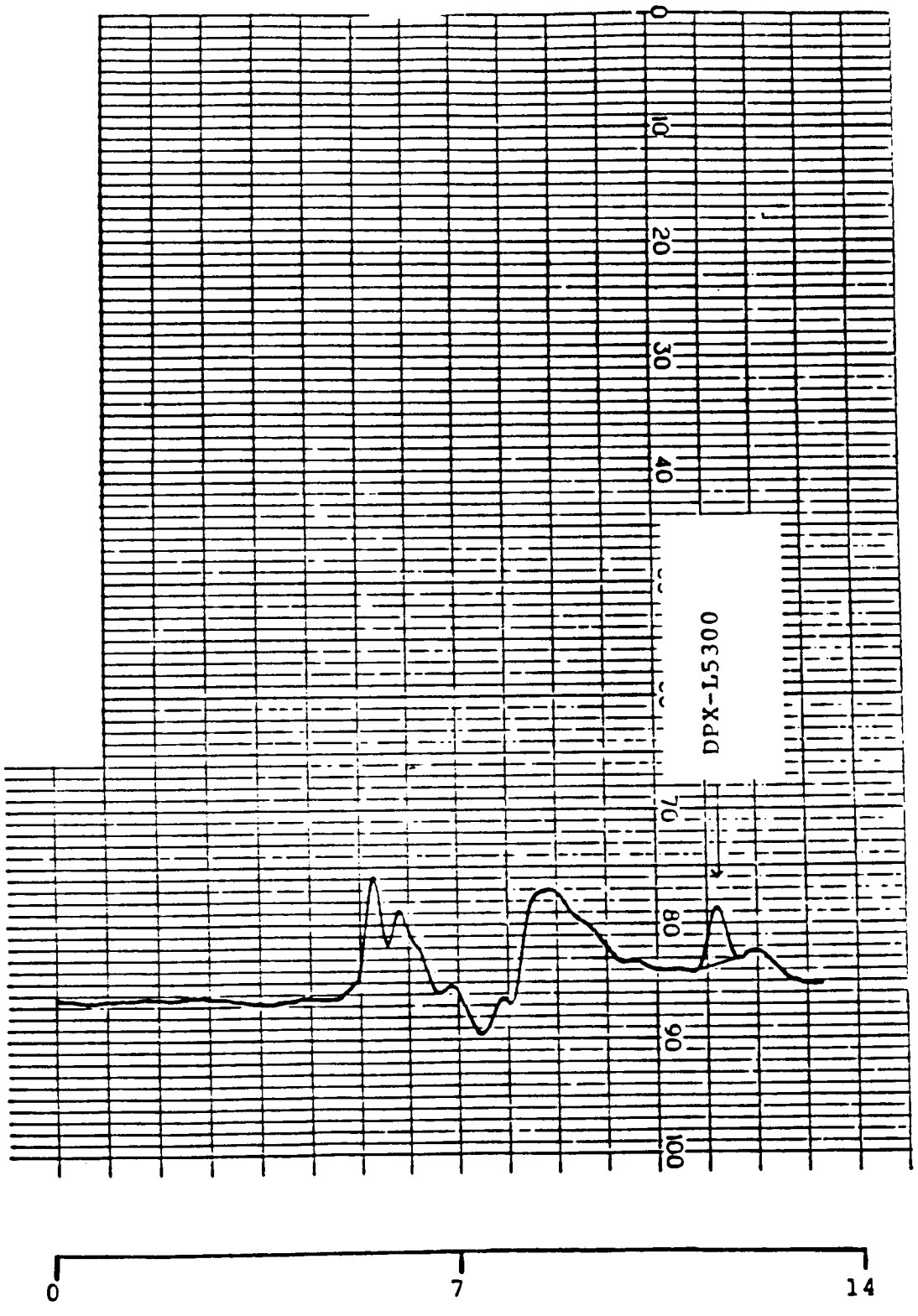


Figure 5. Chromatogram of the wheat straw control sample in Figure 4 spiked at 0.02 ppm (recovery = 91%).

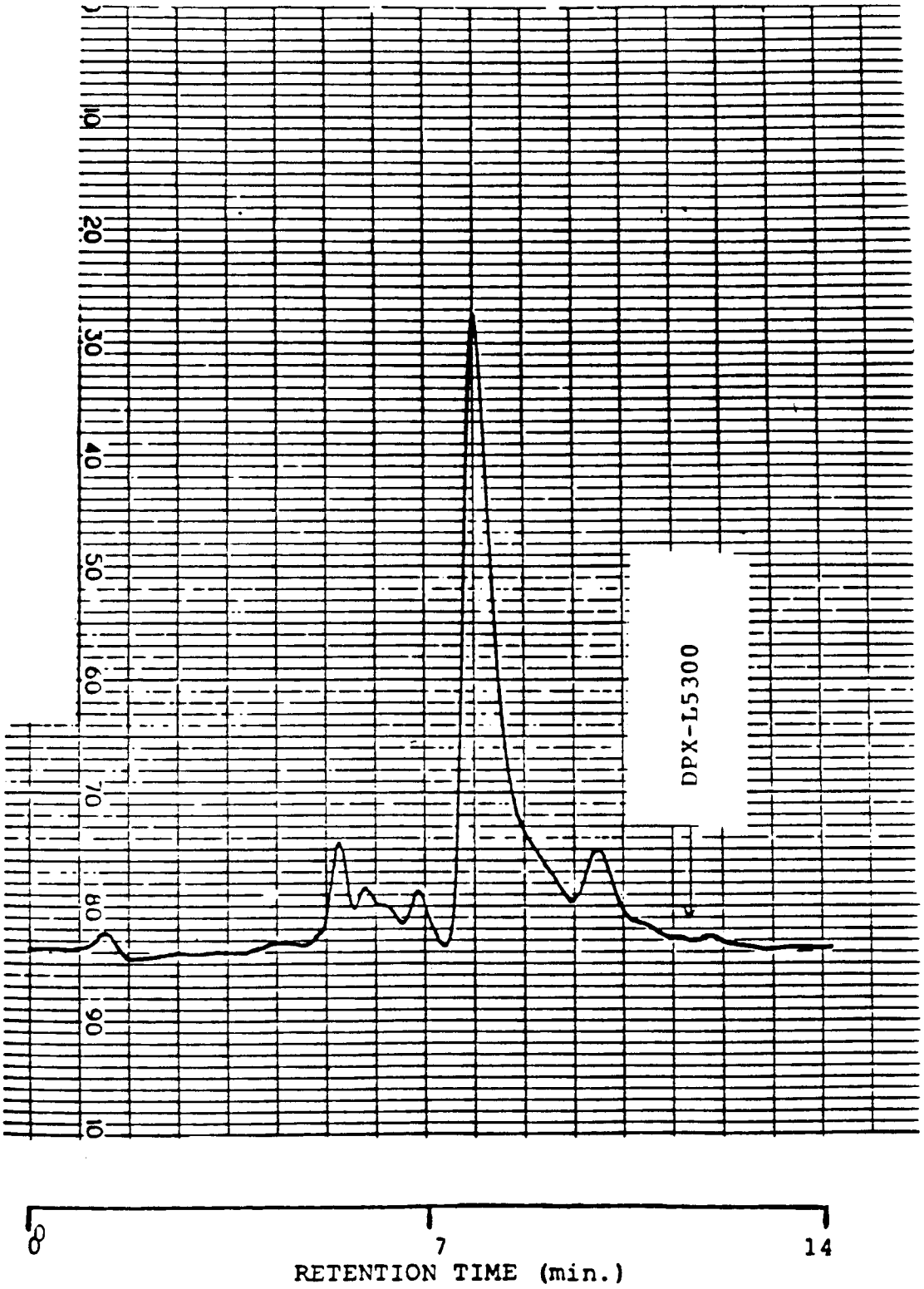


Figure 6. Chromatogram of a treated wheat straw sample (1.0 oz ai/A) from McKenzie, North Dakota.

STORAGE LOCATION OF RAW DATA, REPORTS

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Agricultural Products Department
Experimental Station
Residue Studies Groups' Archives
Wilmington, Delaware 19898